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Title: Ionic mechanisms subserving mechanosensory transduction and neural integration in statocyst hair cells of Hermisenda.

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(NASA-CR-183393) IONIC MECHANISMS  
SUBSERVING MECHANOSENSORY TRANSDUCTION AND  
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# Mechanosensory-Activated and Voltage-Dependent Ion Channels in Statocyst Hair Cells of Hermisenda Crassicornis.

## Description of Research

The neural processing of gravitational-produced sensory stimulation of statocyst hair cells in the nudibranch mollusc Hermisenda has been studied. Our goal in these studies has been to understand how: (a) gravireceptor neurons "sense" or transduce gravitational forces, (b) gravitational stimulation is integrated so as to produce a graded receptor potential, and ultimately the generation of an action potential, and (c) various neural adaptation phenomena which hair cells exhibit arise. Our approach to these problems has been primarily electrophysiological.

Previous research had shown that motile cilia on statocyst hair cells transduce gravitational forces, through active mechanical interaction with statoconia suspended inside the statocyst. The mechanical interaction of the hair cell cilia and the statoconia resulted in a mechanical deformation of the cilia and hair cell membrane, and the opening of ion channels. These ion channel openings result in an influx of cations into the cell and a resulting depolarization.

During the two years of research conducted at Princeton, we studied these mechanosensory-activated ion channels through the use of fluctuation analysis of current "noise" observed under voltage-clamp. We also studied several other classes of ionic channels in the hair cells using voltage- and single-channel patch clamp techniques. These channels (primarily  $K^+$  selective) subserve the hair cells' integrative response to gravitational stimulation. They are activated by changes in the membrane potential of the cell, rather than by mechanosensory stimulation per se.

## Accomplishments

(1) Previous intracellular recording studies of Hermisenda hair cells under current-clamp conditions by other investigators had obtained evidence that the increased voltage-noise and depolarizing generator potential produced by mechanical- or gravitational-induced interaction between hair cell cilia and statoconia reflected mechanosensory-activation of a  $Na^+$ -selective channel. We studied these channels by means of fluctuation analysis of ionic current-noise, under voltage-clamp control. Current noise amplitude was typically 1-2 orders of magnitude greater for hair cells exposed to 1 g force by tilting ("loaded" condition, Figure 1) than in the non-stimulated ("unloaded") case. Current noise amplitude increased in an approximately linear fashion as the steady state holding potential of the hair cell was made more negative than -40 mV (Figure 2). Removal of extracellular sodium resulted in a reversible decrease in noise (Figure 2) amplitude, though it did not completely abolish it. In addition, the power spectra for many cell (~50%) cells was decidedly non-regular with a peak in the 7-12 Hz range. This coincides with the mean frequency of the inherent motions of statocyst hair cells. Individual records of current noise (Figure 2) revealed that the 7-10 Hz peak in the power spectra corresponded to the presence of large, discrete, inward currents, with an extrapolated reversal potential  $> +10$  mV. Collectively, these results indicate that  $Na^+$  ions make a significant contribution to the ionic current through the mechanosensory-activated channels.

(2) Three distinct components of voltage-dependent outward ( $K^+$ ) current were observed under voltage-clamp. Two of these appear to be identical to  $K^+$  currents previously described in detail in other preparations. When depolarized from a

holding potential of -60 mV to potentials more positive than -40 mV, a rapid (~10 ms to peak at 0 mV), transient, 4-aminopyridine (4-AP) sensitive, outward current was observed (Figure 3:  $I_A$ ). Steady-state inactivation of  $I_A$  was studied using a twin-pulse protocol in which the cell was first held at -60 mV, then stepped to a variable potential (from -100 to 0 mV) for 5 sec, before being stepped to a final potential of 0 mV.  $I_A$  was half-inactivated at -43 mV, which is considerably more positive than values typically reported in other cells and implies that  $I_A$  can contribute to the resting membrane conductance of the hair cell. Activation of  $I_A$  was also studied with a twin-pulse protocol. The cell was first stepped to -100 mV, to remove resting inactivation, and then to a variable potential from -60 mV to +20 mV. Activation was steeply voltage-dependent at potentials positive than -40 mV.

(3) A second slower component of outward current, which showed a moderate rate of decay was observed when hair cells were depolarized to potentials more positive than 10 mV. At +20 mV, time to peak for this current was 30-50 ms. This current was blocked by 100 mM TEA, and appears to be similar to the "delayed rectifier" current ( $I_K$ ) seen in other preparations.

(4) With  $I_A$  and  $K_K$  blocked by 5 mM 4-AP and 100 mM TEA, an extremely slow outward current was elicited by depolarization to potentials  $\geq -30$  mV. This current, which showed little inactivation during depolarizing steps as long as 15 sec, increased in amplitude during a sustained depolarization, reaching its steady-state value after 5 sec. Although the kinetics of activation appear similar to those of a calcium activated potassium current ( $I_{K-Ca}$ ) in Hermisenda Type B cells, this current proved to be insensitive to: (1) changes in extracellular  $Ca^{2+}$  concentration over the range of 0-100 mM, (2) addition of calcium channel blockers to the bath ( $Cd^{2+}$ , 5 mM), and (3) intracellular injections of EGTA, a calcium chelator. However, each of these manipulations produced the expected changes in the magnitude of a transient, inward, voltage-dependent (calcium) current. We conclude that the very slow outward current is not a calcium-activated current, although it is most likely a potassium current, since: (1) both peak ( $I_A$ ) and steady-state (measured 500 ms following depolarization) outward current components show the same reversal potential in elevated  $K^+$  solutions (-12 mV in 300 mM  $K^+$ ).  $I_A$  is extremely  $K^+$  selective, and the similar reversal potential for  $I_A$  and the delayed component implies a similar  $K^+$  selectivity. (2) An outward tail current was observed in 4-AP and TEA when the cell was stepped back to -60 mV from command steps more positive than -30 mV, consistent with our estimate of  $E_K$  in 10 mM  $K^+$  ASW (approx. -100 mV).

(5) Single-channel  $K^+$  currents were recorded from Hermisenda hair cells, using the cell-attached patch-clamp recording configuration. Figure 4 depicts several examples of an ~35 pS channel we have seen. Open time distributions indicate that no single exponential decay function will suffice as a fit of the data. This implies the presence of multiple open states. The channel is clearly voltage-dependent and is open a large fraction of the time at potentials more positive than rest (-50 mV). This channel may underlie one of the slower components of macroscopic  $K^+$  current seen in the previous voltage clamp studies.

#### Significance of the Accomplishments

Finding #1, that the current-noise of hair cells increases during gravitational stimulation and that the channels are selectively permeable to  $Na^+$  ions, represents the first direct information concerning the biophysical properties

of mechanosensory-activated ion channels in an invertebrate neuron specialized for graviception.

Findings #2-4, that there are at least three kinetically- and pharmacologically-distinct components of the net outward current elicited by depolarization, represents the first direct analysis of integrative ion currents in an invertebrate graviceptor neuron.

Finding #5, our patch-clamp recordings of single, voltage-dependent  $K^+$  channels in Hermisenda hair cells, represents the first direct single-channel recordings from any graviceptor neuron.

Collectively, our findings provide a detailed and comprehensive view of those ionic channels which subserve the transduction and integration of gravitational stimulation in a primary graviceptor neuron, under normal gravitational conditions.

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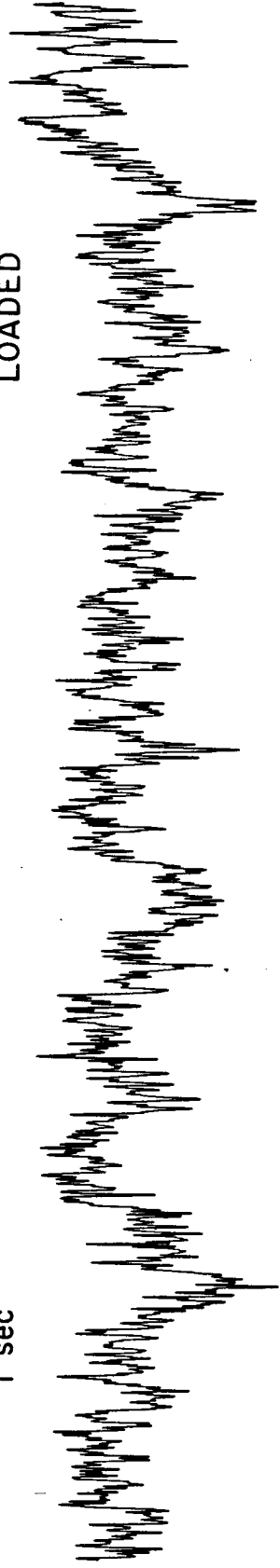
Farley, J. and Rudy, B. Single  $K^+$  channels from rat and Hermisenda brain incorporated into lipid bilayers on patch-clamp pipettes. Biophys. J. Abstrs., 49: 575a, 1986 (abstract).

## Figure Captions

- Figure 1 Current-noise from Hermissenda statocyst hair cell in "loaded" vs. "unloaded" conditions. Cell was voltage-clamped at -60 mV in a standard ASW solution, using conventional two microelectrode voltage-clamp techniques. Current signal was amplified 200 times, filtered at 300 Hz (high-frequency cutoff) and stored on an HP instrumentation recorder for later off-on line analysis. Tilting of preparation resulted in the mass of statoconia coming to rest on or near the caudal portion of the statocyst (loaded condition). Tilting 90 degree in the opposite direction resulted in the statoconia moving towards the opposite pole of the cyst, away from the caudal hair cells (unloaded condition). Note the two conspicuously large bursts of inward (down) current in the loaded condition. These are likely to reflect the simultaneous interaction of a statoconia with one or more motile cilia.
- Figure 2 Effects of  $\text{Na}^+$  ions and holding potential level on current noise amplitude of a partially "loaded" caudal hair cell. All records obtained from same cell. In normal Na (430 mM) ASW (left half) increases in holding potential result in progressively larger current noise variance ( 2-3 times as large as when  $\text{Na}^+$  ions are not present; right half of Figure).
- Figure 3 Transient outward  $\text{K}^+$  current ( $I_A$ ), recorded from statocyst hair cell under voltage clamp. Top: Cell stepped from  $V_H = -60$  mV to +10 mV. Middle: 5 sec. pre-pulse depolarization to -10 mV (+50 command step) inactivates the early transient current without effect upon the slower delayed  $\text{K}^+$  current. Bottom: 2 min later, transient current has returned to full amplitude. Transient current is abolished by 4-AP (2-3 mM) but not TEA (50 mM) (not shown).
- Figure 4 Examples (arrows) of channel currents recorded from Hermissenda statocyst hair cells using cell-attached patch-clamp recording techniques. Depicted here are three successive 50 mS samples of channel activity. Channel openings appear as upward deflections, closings appear as down deflections. The channel opens twice in trace 1, and once in traces 2 and 3. All recordings obtained at the resting potential of the cell ( -50 mV). Slope conductance of the channel was 35 pS. Records filtered at 3 KHz.

0.125 nA   
1 sec

LOADED



-60 mV HOLDING

UNLOADED





ASW

Low  $\text{Na}^+$



- 30 mV



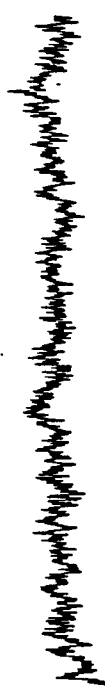
- 40 mV



- 50 mV



- 60 mV



- 80 mV



- 100 mV



0.05 nA

1 sec

